



Miller, V. J., & Stephens, D. J. (2013). Membrane contact sites - an interesting species, an interesting mix. *EMBO Reports*, 14(5), 396-397. <https://doi.org/10.1038/embor.2013.43>

Peer reviewed version

Link to published version (if available):
[10.1038/embor.2013.43](https://doi.org/10.1038/embor.2013.43)

[Link to publication record in Explore Bristol Research](#)
PDF-document

Author version.
Originally published in EMBO reports advance online publication
doi:10.1038/embor.2013.43
Published online: 5 April 2013
<http://www.nature.com/embor/journal/vaop/ncurrent/full/embor201343a.html>

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Membrane contact sites: An interesting species, an interesting mix.

Victoria J. Miller & David J. Stephens*

Cell Biology Laboratories, School of Biochemistry, Medical Sciences Building, University of Bristol,
University Walk, Bristol, BS8 1TD, UK.

*Corresponding author: Tel: 00 44 117 331 2173 Fax: 00 44 117 331 2168 Email:
david.stephens@bristol.ac.uk

Originally published in EMBO reports advance online publication

doi:10.1038/embor.2013.43

Published online: 5 April 2013

<http://www.nature.com/embor/journal/vaop/ncurrent/full/embor201343a.html>

Eukaryotic cells are defined by the presence of membrane-bound organelles that divide the cell into distinct compartments with specialized microenvironments optimized for the reactions taking place in each one. However, membranes pose a barrier to the free diffusion throughout the cell. Transfer between compartments can occur via vesicles or using specialized transfer proteins such as phospholipid transfer proteins. Another solution has evolved in the form of a network of contact sites that link different organelles [1]. These contact sites bring membranes into close proximity (within ~30 nm), tethering them without fusion. Several forms of membrane contact sites have been identified, predominantly connecting the endoplasmic reticulum (ER) with mitochondria, the Golgi, endosomes and the plasma membrane (PM). Membrane contact sites allow for transfer between two compartments, primarily of either calcium ions or lipids, but other functional roles are now emerging. In this issue of *EMBO reports*, Loewen and colleagues reveal a role for plasma membrane-ER contact sites in regulating phosphatidylcholine synthesis in budding yeast [2].

PM-ER contact sites regulate lipid synthesis

In *Saccharomyces cerevisiae* much of the ER lies in close proximity to the PM. This PM-associated ER (pmaER) is enriched with lipid synthesizing enzymes [3] and the contacts between the PM and the ER are thought to allow for cross-talk and regulation of lipid composition of the membranes between the two compartments. Six proteins required for PM-ER contacts in yeast have been identified: the vesicle-associated membrane protein-associated protein (VAP) orthologues Scs2 and Scs22, Ist2 (a member of the TMEM16 ion channel family) and the tricalbin proteins (Tcb1, Tcb2, and Tcb3, orthologues of the extended synaptotagmin family) [4]. Deletion of these genes results in elevated phosphoinositide levels in the PM and induction of the unfolded protein response in the ER, indicating a role for PM-ER contacts in cell signalling and ER function as well as organelle morphology [4].

SCS2 interacts genetically with an integral ER protein of unknown function, *ICE2* [5]. $\Delta scs2\Delta ice2$ cells lack pmaER and show reduced growth, suggesting that the contact sites are important for normal cellular function. In the current paper [2] Loewen and colleagues find that $\Delta scs2\Delta ice2$ cells are choline auxotrophs and show that PM-ER contacts sites are required for phosphatidylcholine (PC) synthesis. PC can be synthesised through the Kennedy pathway from lipid precursors including choline. Addition of choline or overexpression of Opi3, the enzyme that catalyses the last methylation steps of PC biosynthesis, rescued growth of $\Delta scs2\Delta ice2$ cells. Overexpression of the preceding enzyme in the pathway, Cho2, could not rescue the defect indicating a fault in the final methylation steps of PC biosynthesis. Strikingly, although the expression level of the Opi3 protein was similar to wild type cells, Opi3 did not localize to pmaER in $\Delta scs2\Delta ice2$ cells, suggesting a crucial role for PM-ER contacts in regulating the activity of the protein. Scs2 directs localization of the oxysterol binding

protein homologs Osh2 and Osh3 to pmaER [6] and Loewen and colleagues show that Osh3 rescues choline auxotrophy in $\Delta scs2\Delta ice2$ cells but does not restore pmaER. Changes in the abundance of PM-ER contacts are shown to affect PM stability using a detergent-sensitivity assay against the non-ionic detergent NP-40, susceptibility to which is partially rescued by growth on choline-containing media. These data suggest an important role for pmaER in maintaining the structural integrity of the plasma membrane.

The authors then searched for regulators of pmaER by screening for proteins that suppress the choline requirement of the $\Delta scs2\Delta ice2$ mutant and identified the phosphatidic acid phosphohydrolase, Pah1. Surprisingly, a catalytically inactive form of Pah1 also rescues the $\Delta scs2\Delta ice2$ choline auxotrophy, and, unlike Opi3, Pah1 also rescues the PM-ER contact sites. Thus, it appears that Pah1 is involved in establishing pmaER. How this relates to the other structural components of these contact sites remains to be established.

The authors then used an elegant *in vitro* methylation assay to demonstrate that Opi3 can methylate PE to PC *in trans*, a mechanism that had been proposed previously [7] and that offers a pathway for the cell to make rapid changes to membrane composition. As the authors point out, all eukaryotic PE methyltransferases are located on the cytosolic face of the ER while the prokaryotic equivalents are all soluble enzymes. Thus, it is likely that all PE methyltransferases act in *trans* to control PC synthesis in the plasma membrane.

Functions of membrane contact sites

Contact sites between different organelles have recently become the source of considerable interest with roles in controlling organelle morphology [8], regulation of growth factor [9], phosphoinositide [4, 6], and calcium signalling (reviewed in [1]). Others have shown that phospholipids at contact sites can be modified, for example the dephosphorylation of PI4P by Sac1 [6]. Interestingly, Sac1 acts at membrane contact sites that are maintained through binding of Scs2 to the FFAT motif within Osh3. Scs2 maintains pmaER contact in both cases and Osh3 acts as a common adaptor to both Sac1 and Opi3 at pmaER (Fig 1). It is expected that there are common architectural determinants of pmaER that are then used by diverse enzymes to control phospholipid metabolism within the PM.

Localized lipid synthesis at membrane contact sites is also likely to occur elsewhere in the cell. Recent work [1] showed that the small GTP binding protein Rab10 organizes a subdomain of the ER membrane that is rich in the phospholipid synthesis enzyme choline/ethanolamine phosphotransferase 1 (CEPT1) and phosphatidylinositol synthase (PIS) (Fig 1). This domain controls the morphology of the ER, notably through homotypic fusion. Previous work provided evidence for a highly mobile, ER-

derived compartment rich in PIS in which PI is synthesised prior to delivery to other membrane via membrane contact sites [10]. Whether these two phenomena are in fact one and the same and whether local phospholipid synthesis contributes to resolution of homotypic fusion events at the ER remains to be defined. While there is no evidence that PIS or CEPT1 can act in *trans* in the way that Opi3p does, another possibility is that these enzymes could participate in the delivery through flippase activity (Chris McMaster, Dalhousie University, Canada, personal communication).

Perspective

The most interesting aspect of the present work demonstrates the capability for de novo lipid synthesis at membrane contact sites. While the structural components of these membrane contact sites and the enzymes that localize there are beginning to be defined, the purpose of localized lipid synthesis remains unclear. This report opens up many new avenues of investigation. Remaining questions concern the role of Pah1 in establishing pmaER, the mechanisms that regulate Opi3 activity at these sites, and the relationship to other phospholipid synthesis pathways..Further work is also likely to define the role of pmaER in the control of ER stress. Clearly, the nature and function of contact sites between cellular membranes is a hot topic with many questions remaining to be answered.

Acknowledgements Work in the Stephens lab is funded by the BBSRC and MRC. The title is inspired by the Carl Sagan science fiction novel “Contact”.

Conflict of interest The authors declare no conflict of interest.

Figure legend

Fig 1 | Phospholipid synthesis at membrane contact sites. **(A)**. PC synthesis mediated by the PE N-methyltransferase, Opi3p, acting in *trans* at ER-PM contact sites. **(B)** Sac1 catalyses the dephosphorylation of PI4P to generate PI also at ER-PM contact sites. **(C)** In metazoans, Rab10 mediates the localization of CEPT1 and PIS to dynamic ER domains that act in phospholipid synthesis and control ER morphology. Evidence also suggests a role for this domain at ER-PM contact sites. PE, phsophatidylethanolamine; PME, phophatidylmethylethanolamine; PC, phosphatidylcholine; PI4P, phosphatidylinositol-4-phosphate; PI, phosphatidylinositol; PH, plekstrin homology; PIS, phosphatidylinositol synthase; CEPT1, choline/ethanolamine phosphotransferase 1; FFAT, two phenylalanines in an acidic tract.

References

1. Helle SC, Kanfer G, Kolar K, Lang A, Michel AH, Kornmann B (2013) *Biochim Biophys Acta in press* doi: 10.1016/j.bbamcr.2013.01.028
2. Tavassoli S, Chao JT, Young BP, Cox RC, Prinz WA, de Kroon AI, Loewen CJ (2013) *EMBO Rep in this issue*
3. Pichler H, Gaigg B, Hrastnik C, Achleitner G, Kohlwein SD, Zellnig G, Perktold A, Daum G (2001) *Eur J Biochem* **268**: 2351-2361
4. Manford AG, Stefan CJ, Yuan HL, Macgurn JA, Emr SD (2012) *Dev Cell* **23**: 1129-1140
5. Loewen CJ, Young BP, Tavassoli S, Levine TP (2007) *J Cell Biol* **179**: 467-483
6. Stefan CJ, Manford AG, Baird D, Yamada-Hanff J, Mao Y, Emr SD (2011) *Cell* **144**: 389-401
7. Janssen MJ, de Jong HM, de Kruijff B, de Kroon AI (2002) *FEBS Lett* **513**: 197-202
8. Friedman JR, Lackner LL, West M, Dibenedetto JR, Nunnari J, Voeltz GK (2011) *Science* **334**: 358-362
9. Eden ER, White IJ, Tsapara A, Futter CE (2010) *Nat Cell Biol* **12**: 267-272
10. Kim YJ, Guzman-Hernandez ML, Balla T (2011) *Dev Cell* **21**: 813-824

Victoria J. Miller is a postdoctoral researcher in the School of Biochemistry at the University of Bristol, Bristol, UK.

David J. Stephens is a Professor of Cell Biology at the University of Bristol, Bristol, UK.

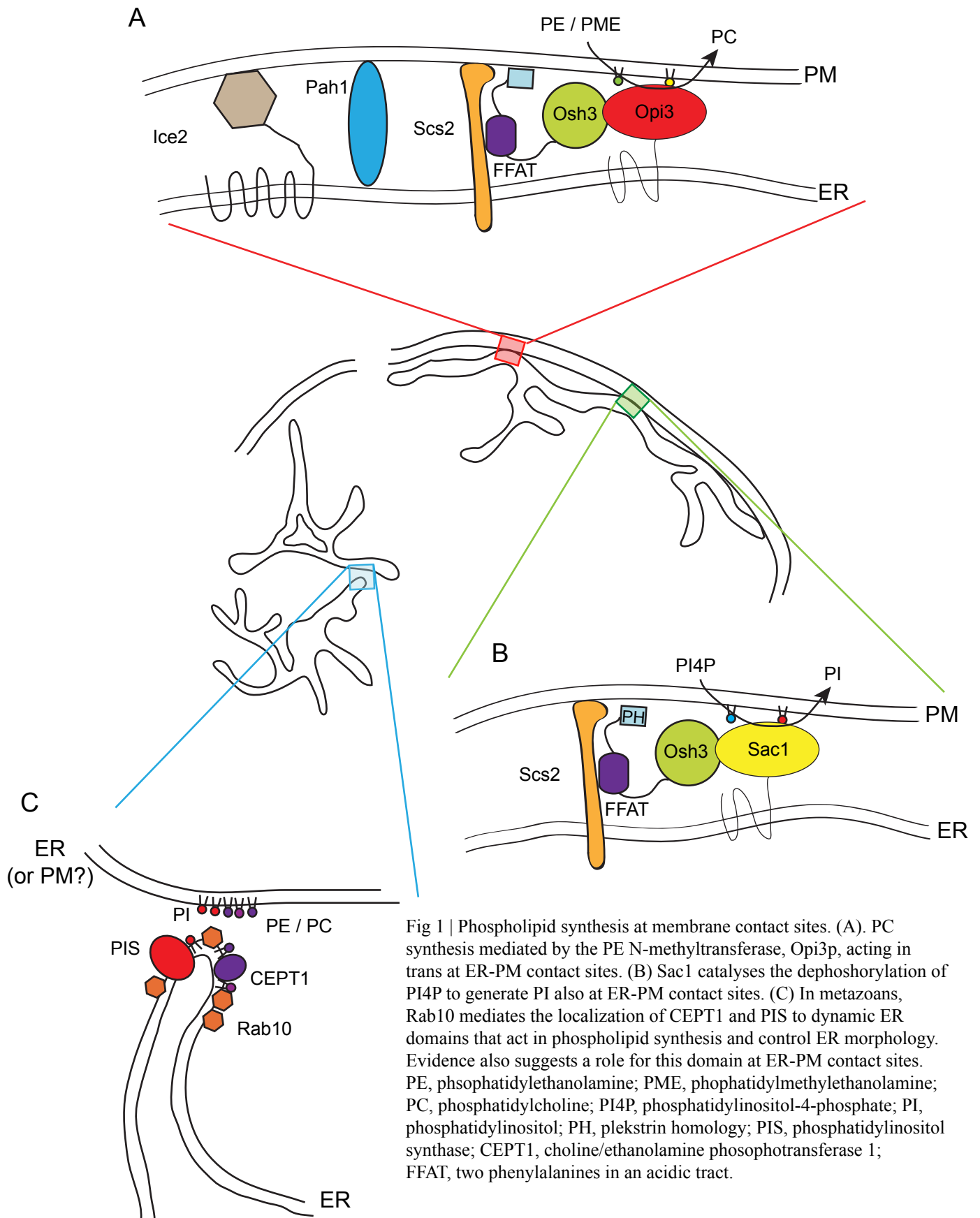


Fig 1 | Phospholipid synthesis at membrane contact sites. (A). PC synthesis mediated by the PE N-methyltransferase, Opi3p, acting in trans at ER-PM contact sites. (B) Sac1 catalyses the dephosphorylation of PI4P to generate PI also at ER-PM contact sites. (C) In metazoans, Rab10 mediates the localization of CEPT1 and PIS to dynamic ER domains that act in phospholipid synthesis and control ER morphology. Evidence also suggests a role for this domain at ER-PM contact sites. PE, phosphatidylethanolamine; PME, phosphatidylmethylethanolamine; PC, phosphatidylcholine; PI4P, phosphatidylinositol-4-phosphate; PI, phosphatidylinositol; PH, plekstrin homology; PIS, phosphatidylinositol synthase; CEPT1, choline/ethanolamine phosphotransferase 1; FFAT, two phenylalanines in an acidic tract.